A CHROMATOGRAPHIC INVESTIGATION OF THE FATTY ACID CONTENT OF 12 WILD SPECIES OF GOSSYPIUM

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CHAPTER I

INTRODUCTION

The morphological characters of both plants and animals are the primary criteria of their classification. They also furnish the clues to phylogenetic relationships.

There are no totally reliable taxonomic characters free from the effect of adaptive modification. Structure patterns of plants and animals depend on individual survival values which are the products of their adaptive properties. Consequently, clear, precise and phylogenetically sound classifications must be based on characters that are practically invariable, and are essentially immune to modification Sibley (1960).

The accepted classification of genus Gossypium developed by Hutchison et al. (1947) is likewise based on morphological, genetic, and cytological differences. Systematic botanists accept the classification of Hutchinson et al. (1947) as a reasonable and natural account of plant speciation.

In recent years, attempts have been made in order to find chemical differences and similarities to verify and develop further the classifications of several species which are based on morphological grounds. This approach has been made possible through the development of partition chromatography.

Since all species of Gossypium have high oil content in the seed,

the fatty acid contents of the oils would seem to be a logical place to check for chemical differences in the species of the genus.

The objectives of this investigation were:

1. To determine whether the species differ in the fatty acid composition of their oils.

2. If differences are found, to determine whether the similarities and the differences in the comparative fatty acid contents of the oils are correlated with the classification based on morphological, genetic, and cytological data.

In this study, the fatty acid content of the oil from 12 species of wild Gossypium from the sections: Stocksiana, Klotzschiana, Erioxyla, Thurberana, and Anomala, was analyzed by Gas-liquid-chromatography.

CHAPTER II

REVIEW OF LITERATURE

This review is limited to the distribution of the lintless uncultivated wild species of cottons, the evolutionary relationships of the twelve species on which this study was conducted, and the use of biochemical methods for investigating the evolutionary relationships of various plants and animals.

Relationships of the Species Under Study

Saunders (1961) described the wild lintless species of genus Gossypium as perennial xerophytic shrubs which occur in the arid regions of the tropics and subtropics. Some of the species are drought resistant.

The studies of Skovsted (1935), Beasly (1942) have shown that the basic chromosome number of the genus Gossypium is 13. All species have either 13 or 26 chromosome pairs. The species with 13 chromosome pairs fall into five major groups between which the chromosome homology is low. These groups are: (1) The wild Australian species, (2) the wild Arabian and African species, (3) the wild species of Northern India, Arabia and Somaliland, (4) the wild American species, and (5) the cultivated species of the old world.

The cottons with 26 chromosomes carry a complement of chromosomes which consists of a set of 13 chromosomes homologous with the genomes of the cultivated old world species and a set of 13 chromosomes homologous

with those of the wild lintless American species.

Hutchison (1954) stated that it is almost certain that central Africa was the center of origin of the young genus Gossypium at a time when climatic and geophysical conditions were different from today. Saunders (1961) assumed that the present occurrence of the wild American species and the wild Australian species in the Continents of America and Australia respectively, in addition to the presence of other wild species of Gossypium in Asia and Africa would find an acceptable explanation in terms of Wegener's theory of Continental Drift.

Evolutionary Relationships

Silow (1944) pointed out in his study of the species that there is, on the whole, so little cytological differentiation between the species that the species differences must be attributed mainly to genetic bases. He pointed out increasing differentiation through the building up a genetic barrier between the genotypes of isolated groups. Where there is an exchange of genes between one group and another, there is, on the other hand, an equal elimination of the new interspecific hybrids, through the malfunctioning of the genes in the new combinations. Thus groups between which such barriers exist are regarded as specifically distinct groups.

The evolutionary relationships between the wild American Lintless species of Gossypium have been carefully reviewed by Hutchinson et al. (1947), and Saunders (1961). They report the following relationships:

G. klotzschianum and G. davidsonii of Section Klotzschiana intercross freely and rapidly, giving a fertile complement of normal seed in the F_1, first, second, and third generation hybrid plants were found to be normal and fully fertile. In addition to fertility, it was found that

the range of segregation in the F_2 covers the spectrum between the parental types but does not extend beyond them/

Any cross between G. raimondii and G. harkensii of Section Erioxyla produced empty seeds.

G. raimondii X G. aridum (Section Erioxyla) gave only one capsul and showed evidence of one successful fertilization out of 34 pollinations.

G. raimondii, morphologically resembles G. Klotzschianum and upon this basis it was placed in Klotzschiana, but as far as the crossing behavior of G. raimondii is concerned, it is sometimes grouped either in Erioxyla or Thurberana because it crosses fairly easily with G. thurberi of Thurberana, and G. armorianum of Erioxyla, but most of the seeds were empty or partially filled. In the cross of G. raimondii X G. thurberi, only 10% of the F_1 seeds germinated and produced hybrids; and the F_1 plants were only partially fertile.

None of the seeds of F_1 of G. raimondii X G. armorianum, germinated. It was concluded that there is sufficient evidence to include G. raimondii with G. klotzschianum and G. davidsonii in Klotzschiana on both its crossing behavior with G. davidsonii and on its morphological resemblance with with G. klotzschianum.

G. thurberi (Section Thurberana) crosses rather easily with G. armorianum, and G. harkensii of Section Erioxyla, giving F_1's which are 85%, and 70% self fertile, respectively. Also a small F_2 population of G. thurberi X G. armorianum was highly variable in both morphology and fertility.

In the F_1 of the G. thurberi X G. aridum crosses, about half of the flowers pollinated, but nearly all the seeds obtained were empty. A single F_1 when used in back corss with the parental species this F_1 had

low fertility.

The species G. armorianum and G. aridum of Section Erioxyla cross with difficulty and the F_1 is very highly sterile.

G. armorianum crosses freely with G. harkensii of Section Erioxyla, giving a vigorous, fully fertile F_1, and very variable F_2 with some plants as fertile as the F_1 plants.

G. lobatum was considered by Saunders (1961) to resemble G. ardium in all the aspects and it was grouped together with G. aridum, G. armorianum and G. harkensii in Section Erioxyla.

In the study of the chromosome pairing and the degree of relationship between the wild lintless American species of cottons, Hutchinson et al. (1947) concluded that in all crosses between the American wild species, and in all F_1's that have been grown to flowering, the chromosome pairing approaches 13 bivalents. No evidence of cytological differentiation was found. It was concluded that G. thurberi of Thurberana must be regarded as the central type, and that its closest relations are G. armorianum and G. harkensii of Erioxyla.

G. aridum of Erioxyla was found to be genetically isolated, but nearer to G. thurberi of Thurberana, G. armorianum and G. harkensii of Erioxyla than to G. raimondii, supposedly of Klotzschiana.

G. raimondii is also isolated but appears to be a little closer to G. thruberi than to the other American lintless wild species of cottons.

G. klotzschianum and G. davidsonii are more isolated and only on morphological, and geographical grounds can they be regarded as closer to G. raimondii than to the other species.

Saunders (1961) reviewed the evolutionary relationships between G. anomalum of Section Anomala, and the species of the other sections. He

concluded that the hybridization was successful with G. davidsonii of Klotzschiana, while the hybridization of G. anomalum with the G. somalense and G. longicalyx of Section Stocksiana gave good seeds but the F_1 plants were apparently sterile.

G. somalense of Stocksiana was found to give vigorous hybrids with G. anomalum of Anomala.

G. longicalyx, which was recently recognized by Saunders (1961) and placed in the Section Stocksiana, gave only a few seeds in crosses with G. klotzschianum and G. anomalum of Anomala, and the hybrid of G. longicalyx X G. klotzschianum was vigorous but had not flowered at the time of publication.

A summary of the inter-relationships, and the characters of the F_1 hybrids of the species under study is as follows:

Genome	F_1 characters
G. thurberi X G. aridum	apparent sterility with empty seeds
X G. raimondi	apparent sterility with empty seeds
X G. armorianum	high degree of self fertility
X G. harkensii	high degree of self fertility
G. armorianum X G. raimondii	produced non germinating seeds
X G. aridum	highly sterile
X G. harkensii	vigorous and fully fertile
G. raimondii X G. harkensii	produced empty seeds
X G. aridum	vigorous and sterile
X G. davidsonii	produced empty seeds
G. davidsonii X G. klotzschianum	high degree of fertility
G. anomalum X G. davidsonii	Successful crossing but no seeds
X G. somalense	few seeds with apparent sterility

G. anomalum	X G. klotzschianum	produced few seeds
	X G. longicalyx	few seeds with apparent sterility
G. longicalyx	X G. klotzschianum	vigorous plant

The Use of Chemotaxonomic Methods of Classification:

The partitionchromatographic method of Martin and Synge (1941) and its adaptation by Condsen et al. (1944). has been a useful method for qualitative analysis of known and unknown chemical extracts of both plant and animal tissues. Since the method is able to quantitatively measure very small amounts of many compounds, considerable interest in using it as a taxonomic tool has developed. Various degrees of success have been obtained in attempting to differentiate various species on the basis of chemical differences.

In studies of phenolic compounds in the leaves of Lotus (Leguminosae) Harney and Grant (1964) found by chromatographic methods that the distribution of these substances complement the classifications developed by cytotaxonomical methods.

Using chromatographic methods in a study of various compounds of the root tip of nine species of 4 genera of the Iridaceae, Riley and Bryant (1961) are able to show that the chromatographic patterns of species of different genera differ more than those of the same genus.

Torres and Levin (1964) in a survey of the seven taxa of the subgenus Diplothrix (Zinnia - composiate), and several artificial hybrids, showed that relationships established by chemotaxanomic methods agreed in each instance with those established by other approaches. In addition, the chromatographic data provided a new insight into the relationships among the species of the Diplothrix diplo-polyploid complex. The results

substantiated the previously determined genomic relationships, and supported the hypothesis of the ancestry of diplo-polyploid complex.

Smith and Abashian (1963) used the chromatographic methods to determine the alkaloidal contents of the following: (1) all 52 nicotiana species representing all taxonomic sections and centres of geographical distribution; (2) 35 two-species combinations including one species of hybrid origin, 5 F_1 interspecific hybrids, 24 amphiploids, and 5 sesquiploids and (3) 14 three-species combinations, including 6 hybrids between an amphiploid and a third species and 4 different 3-species combinations with doubled chromosome number. No simple basis of inheritance for the alkaloidal contents was evident. In addition, there were no clearly defined associations between phylogenetic positions and the alkaloid contents observed.

In a study of the extracts from mature leaves of 21 varieties of mango, Teas et al. (1959) found that 7 varieties could be distinguished from all the others, the other 14 varieties fell into 4 groups of 2 - 5 varieties each. They concluded that this chemical technique might be an aid in the identification of varietal differences in mangoes.

With paper chromatography, Rice (1964) obtained partial success in distinguishing varieties of grape through their differences in anthocyanin pigment contents.

Kirk et al. (1954) reported that paper chromatography could be used to differentiate several, morphologically similar species of land snails representing 7 species from 4 genera, however, they had not yet identified the components involved, but the patterns for each species was distinctive for each species. They concluded that the paper chromatographic analysis of simple tissue extracts offer a tool which is likely

to be of considerable value to taxonomists.

Thompson (1960) was able to identify different species of fish, through the technique of moving boundary electrophories of water extracts of the fillets.

In an electrophoretic study of the egg-white proteins of 23 breeds of the domestic fowl (Gallus gallus) selected for morphological and physiological diversity, Sibley and Johnsgard (1957) found no variation in the egg-white proteins among these breeds. They concluded that the structure of the egg white proteins is a phylogenetically conservative character for the different breeds of Gallus gallus.

In summary considerable genetic variation exists in the relationships between the wild species of cottons of the same section and of the different sections as well. Cytogenetically, it was found that it is impossible to draw lines between the sections or even between the species. Consequently, chemical analyses might be useful in an attempt to further understand these species' relationships. In a number of cases studied, the chromatographic analyses of the chemicals were found to be valuable procedures in the study of the phylogenetic relationships, taxonomy, and genetics of both plants and animals.

CHAPTER III

MATERIALS AND METHODS

Species:

The following twelve wild lintless species of Gossypium were obtained from the United States Department of Agriculture: G. aridum, G. armorianum, G. harkensii, G. lobatum, G. klotzschianum, G. davidsonii, G. raimondii, G. thruberi, G. gossypioides, G. anomalum, G. somalense, and G. longicalyx. The first nine species are native to America and represent sections Erioxyla, Klotzschiana, and Thurberana. G. somalense and G. longicalyx are old world species which represent Section Stocksiana. G. anomalum represents Section Anomala.

The seed used was grown at Tguala, Mexico in 1960 and was provided by the Cotton and Cordage Fibers Research Branch, United States Department of Agriculture.

Oil Extraction:

The following procedure of oil extraction was used: A quantity of seeds of each species was ground with a household type food grinder. The samples were stored in closed jars at (0-(-1)) C° until the oil was extracted. The oil of 15-gram samples was extracted with a Soxhlet extractor using hexane as solvent. After the extraction was complete, each flask was detached, and the solutions were concentrated and centrifuged to remove the impurities. The excess hexane was then completely removed and the net weight of the oil was obtained. The residues were

re-extracted with fresh hexane in order to insure complete removal of the oil from the ground seeds.

Sample Preparation of Fatty Acid Methylesters:

1. Esterification: To separate various fatty acids of each oil by Gas-Liquid-Chromatography, it is necessary to form the methyl esters of the fatty acids of each oil. The conversion of the fatty acids to their methyl esters was accomplished by the interesterification method described by Mason et al. (1964). Basically the procedure is as follows: Approximately 200 milligrams of the oil from each sample was weighed and placed into 25-milliliter, glass stoppered Erlenmyer flasks. The following reagents were then added to the oil in the following sequence:

1. 10.0 milliliters of benzene
2. 4.0 milliliters of DMP (dimethoxy propane)
3. 5.0 milliliters of methanol
4. 1.0 milliliters of 2.0 N sodium methoxide

The mixture was swirled and allowed to stand at room temperature for 5 minutes.

5. 0.69 milliliters of "HCl in methanol." The resulting mixture was swirled again and allowed to stand for 50 minutes.

6. 2.0 grams of previously prepared solid neutralizer of sodium bicarbonate ($NaHCo_3$), sodium carbonate (Na_2Co_3), and sodium sulphate (Na_2So_4), in the proportion 2:1:2 respectively, was added to the mixture. The mixture was swirled periodically during a 30-minute period and let stand for not less than 12 hours.

7. The supernatants were decanted into 25 milliliter, volumetric flasks, the residues were washed twice with 2 milliliters of methanol and the mixture was let stand, after decanting into the volumetric

flasks. The volumes of the prospective fatty acid methyl esters were adjusted to 25 milliliters by adding more methanol to the volumetric flasks. The fatty acid methyl esters were kept in a refrigerator for the future chromatographic analysis.

Chromatographic Method:

The instrument used for the analyses of the fatty acid methyl esters mixture was a Perkins Elmer 800 Gas Chromatography. The instrument was equipped with a hydrogen ionizing detector, and an eighteen-step Attenuator with ranges from X1 to X500 K.

The hydrogen gas, which is necessary for the operation of the flame ionization detector, was of laboratory grade.

The air, which is necessary for producing a mixture with the hydrogen was of laboratory grade.

A Bristol's Dynamaster recorder with 0.05 to 0 to 1.05 MV range, 0 to 100 scale chart paper, and $\frac{1}{2}$ inch per minute chart speed was used for recording the eluted fatty acid peaks.

The column material was 14.5% of E. G. S. (ethylene glycol succinate) on Anakrom, 100 to 110 mesh, type A, was packed in $\frac{1}{4}$" X 6' aluminum column.

The fatty acid methyl esters were run under the following conditions:

1. The column temperature begins at 85°C and ends at 195°C.

2. The carrier gas was the nitrogen with a flow rate of 60 cubic centimeters/minute.

3. The hydrogen flow to the detector was regulated by the pressure gage at 18 pounds/square inch.

4. The air flow rate to the instrument was regulated by the pressure gage to 40 Ps/inch.

5. An attenuation range between 2x - 500 x was used for detecting the expected fatty acid peaks. The sample inlet temperature was 290 - 310° C.

A micro liter syringe of 10 microliter size (manufactured by Hamilton Co. inc., Whittier, Calif.) was used to inject a size of 3 micro liter of the standards and the samples of the fatty acid methyl esters.

The chromatograms of the methyle esters standards and the samples were obtained. The fatty acids of each sample were represented by their peaks and were identified by means of comparing the retention times of each peak of the standards with those of the unknown peaks of each sample.

The retention time is the interval in minutes between the sample injection and the elution of the samples peaks.

The Calculations:

1. The percentage of the oil component of each species in the first run of oil extraction was calculated by using the following simple formula:

$$\frac{\text{the net weight of the oil}}{\text{the weight of ground cotton seeds}} \times 100$$

2. The percentage of the oil component of the ground cotton seeds' residues was calculated by using the following formula:

$$\frac{\text{the net weight of the reextracted oil}}{\text{the weight of the cotton seeds' residues}} \times 100$$

3. For calculating the fatty acid components quantitatively, the following method was used:

a. The number of u moles of the standard components under each peak was determined by calculating the concentration of each fatty acid in the standard and multiply by the amount injected (microliter)

$$\frac{\text{u moles}}{\text{U L.}} \quad \text{X U L.} = \text{U moles under each peak}$$

b. The area under each peak for the Standards and for the samples was determined and the number of U moles per unit area of the fatty acid of the Standard was determined by using the following formula:

$$\frac{\text{u mole total under each peak}}{\text{the area of each peak}} = \text{u mole per unit area}$$

c. The number of u moles under each peak of the fatty acid of the samples was determined by multiplying the moles per unit area by the area of the peaks corresponding to those of the known compounds.

d. The u moles of the fatty acids of each sample were added together and then by using the following equation we were able to get the u mole % of each fatty acid in the sample.

$$\frac{\text{individual fatty acid in u moles}}{\text{total u moles of fatty acids in the sample}} \quad \text{X 100}$$

CHAPTER IV

RESULTS AND DISCUSSION

The results of the quantitative analyses of the fatty acids of the 12 species of the wild lintless cottons are presented in Table I.

Table II presents the comparative fatty acid contents of the species under study.

Table III indicates the species where each fatty acid was found in large quantity.

Seven fatty acids were detected in the species G. armorianum, G. lobatum, G. harkensii, G. aridum, G. gossypioides, G. thurberi, G. raimondii, G. klotzschianum, G. davidsonii, G. longicalyx, G. somalense and G. anomalum. No species contained all seven of the fatty acids.

Four fatty acids were found in every chromatogram of the twelve species. These fatty acids were: palmatic acid, stearic acid, oleic acid and linoleic acid. In addition when the fatty acids linolineic and archidic do appear, with some other unidentified acid, they occur in only trace amounts.

Study of the Sections: Erioxyla, Klotzschiana and Thurberana

The chromatographic patterns of the distribution of the fatty acids of the oil of 4 species of the American diploid were qualitatively similar. These species are G. davidsonii, G. raimondii, G. lobatum and G. armorianum. These four species possessed palmatic acid, stearic acid, oleic acid, and linoleic acid, but appear to lack myrsitic acid. The absence of the

TABLE I

QUANTITATIVE CONTENTS OF THE FATTY ACIDS AND GLYCEROL MEASURED IN U MOLE %

Species	Glycerol	Myrsitic Acid C_{14}	Palmatic Acid C_{16}	Stearic Acid C_{18}	Oleic Acid C_{18}^{-}	Linoleic Acid $C_{18}^{=}$	Linolenic Acid $C_{18}^{=-}$	Arachidic Acid C_{20}
G. thurberi	18.21	8.08	26.25	3.22	12.81	31.40	-	-
G. gossypioides	3.66	38.47	23.57	1.45	7.98	24.83	-	-
G. klotzschianum	22.29	4.51	26.54	4.51	14.80	26.99	0.32	-
G. davidsonii	13.60	-	26.70	5.00	23.92	30.40	-	0.3
G. raimondii	24.57	-	22.25	3.32	17.22	17.22	0.03	0.2
G. aridum	27.76	4.68	31.22	3.09	13.68	19.08	0.27	0.17
G. harkensii	8.47	1.91	26.58	2.70	33.45	25.93	0.92	-
G. Lobatum	23.63	-	30.05	4.96	13.04	28.12	0.15	-
G. armorianum	22.27	-	24.03	4.51	26.42	22.54	-	0.20
G. somalense	30.05	-	32.58	5.83	11.60	19.25	1.52	-
G. longicalyx	6.72	7.76	27.49	3.41	18.40	36.18	-	-
G. anomalum	18.72	4.24	31.85	2.78	12.67	29.33	0.37	-

TABLE II

QUALITATIVE* CONTENTS OF THE FATTY ACIDS OF THE TWELVE SPECIES

Species	Myrsitic Acid C_{14}	Palmatic Acid C_{16}	Stearic Acid C_{18}	Oleic Acid C_{18}	Linoleic Acid $C_{18}^{=}$	Linolenic Acid $C_{18}^{=}$	Arachidic Acid C_{20}
G. thurberi	X	X	X	X	X		
G. gossypioides	X	X	X	X	X		
G. klotzschianum	X	X	X	X	X	t	
G. davidsonii		X	X	X	X		t
G. raimondii		X	X	X	X	t	t
G. aridum	X	X	X	X	X	t	t
G. harkensii	X	X	X	X	X	t	
G. lobatum		X	X	X	X	t	
G. armorianum		X	X	X	X		t
G. somalense		X	X	X	X	t	
G. longicalyx	X	X	X	X	X		
G. anomalum	X	X	X	X	X	t	

*X = The fatty acid was found in high quantity

t = The fatty acid was found in trace amount

TABLE III

QUALITATIVE CONTENTS OF THE FATTY ACIDS OF THE TWELVE SPECIES WHICH APPEAR IN HIGH QUANTITY

Species	Myrsitic Acid C_{14}	Palmatic Acid C_{16}	Stearic Acid C_{18}	Oleic Acid C_{18}^{-}	Linoleic Acid $C_{18}^{=}$
G. thurberi	X	X	X	X	X
G. gossypioides	X	X	X	X	X
G. klotzschianum	X	X	X	X	X
G. davidsonii		X	X	X	X
G. raimondii		X	X	X	X
G. aridum	X	X	X	X	X
G. harkensii	X	X	X	X	X
G. lobatum		X	X	X	X
G. armorianum		X	X	X	X
G. somalense		X	X	X	X
G. longicalyx	X	X	X	X	X
G. anomalum	X	X	X	X	X

linolenic and arachidic acids from some of these four chromatograms perhaps was due to the mere failure to detect extremely small quantities of these acids in certain species due to insufficient sensitivity of the procedure. It can be concluded that the linolenic acid and arachidic acid occur in too small quantities to be dependable characters. Consequently, they are excluded in this study.

The quantitative analyses of the fatty acid contents of these four species were close to general agreement; however this does not justify them as a criteria of classification, since the fatty acid content varies as a result of sampling, the columns used, and other conditions in the instrument Mason et al. (1964). Other workers who have conducted similar studies have used qualitative identification of the chemical components as a criteria for classification, and for establishing phylogenetic relationships. Harney and Grant (1964), Kirk et al. (1954), Rice (1964), Smith and Abashian (1963), Teas et al. (1959).

The striking similarity between the chromatographic patterns of these four species suggests that there is a close relationship between them.

A comparison between the systematic grouping of the four species and their chemical similarity, it can be stated that the species of sections Erioxyla and Klatzschiana; G. lobatum and G. armorianum of Erioxyla, G. davidsonii, and G. raimondii of Klotzschiana were the only species which possess this chromatographic pattern. Though there is a similarity in the patterns, there is no reason to suggest that the chemical similarity is limited to the species of either Erioxyla or Klotzschiana, since the agreement was partial, and it was found that the species, G. armorianum and G. lobatum of section Erioxyla showed a similar chemical pattern to G. davidsonii and G. raimondi of Section Klotzschiana. When the chromato-

graphic patterns of G. thurberi, G. gossypioides, G. aridum, G. harkensii and G. klotzschanum are compared, the fundamental patterns were the same for all these five species, and as the patterns indicate, the fatty acids contents of the oil of each species were myrsitic acid, palmatic acid, stearic acid, oleic acid, linoleic acid. The quantitative analyses of the fatty acids of these five species show a significant variation, however. G. thurberi, G. klotzschianum and G. aridum were quanititatively similar. G. gossypoides and G. harkensii appear to be rather distinct. G. gossypiodes is characterized by a comparatively high myrsitic acid content and low oleic acid content. G. harkensii on the other hand has high oleic and low myrsitic acid. This apparent quantitative variation may be attributed to the changes of the columns of the gas-liquid-chromatography apparatus, since we ran the first three species in one column, and G. gossypioides and G. harkensii in another column. So as before we dropped the quanititttive variation between the fatty acids of different species, and the presence of the trace fatty acids, from our consideration in this study.

According to the chromatographic pattern similarity and on the qualitative content of the fatty acids we suggest that there is a close relationship between G. thruberi, G. aridum, G. gossypioides, G. klotzschianum and G. harkensii on the basis of the biochemical analyses of their fatty acids.

In these five species, G. thruberi and G. gossypiodes represent Thurberana, G. aridum and G. harkensii reprsent Erioxyla, and G. klotzschianumrepresent Klotzschiana. According to the biochemical analyses of their fatty acids we suggest that there is a close relationship between these five species. Within this group and according to the results of

this analyses we find that G. thurberi and G. gossypioides of Thurberana show a close relationship between each other, which in turn was in consistence with their grouping on morphological basis. G. aridum and G. harkensii also show a close relationship between each other and this relationship was consistent with their grouping on morphological basis. However, there is no reason to say that the results of biochemical analyses were in agreement with the grouping of Hitchison et al. (1947). Since included five species which represent the Sections Erioxyla, Klotzschiana, and Thurberana. We can say that the closeness between G. thurberi and G. gossypioides on one hand, and between G. aridum and G. harkensii on the other hand was not because of their morphological similarities since these four species show the same similarity between each other along with G. klotzschianum.

Study of Section Anomala

G. anomalum is a species of Section Anomala of the wild African lintless species Hutchison et al. (1947), was the only species of this section used in this study. The species is included to compare its pattern with those of the eleven species in the other 4 sections. The chromatographic pattern of G. amomalum shows the peaks of myrsitic acid, palmatic, stearic, oleic acid, linoleic acid and linolineic acid. The last peak appeared in very trace amounts as usual.

Study of Section Stocksiana

G. longicalyx and G. somalense were taken in this study as a representative of Stocksiana of the Asian and African lintless wild species.

The chromatographic patterns of these two species differ markedly from each other in that G. longicalyx is comparatively high in myrsitic and linoleic, while G. somalense is low in linoleic acid. In addition,

G. somalense does not contain myrsitic acid.

G. longicalyx was placed in section Stocksiana with G. somalense largely on geographical and morphological affinities, Saunders (1961). The chrotographic patterns of these two species suggest that relationship between the G. longicalyx and G somalense is not close.

Study of the Biochemical Relationship of the 12 Species of the Five Sections of the Genus Gossypium

The object of this study was to elucidate the interrelationship between the species of the genus Gossypium. The following results were found after the proper comparison of the chromatographic patterns of the 12 species, after exclusion of the quantitative variation between the fatty acids of these species, and deleting of linolineic acid $C_{18}^{=}$ and arachidic acid C_{20} because of their minute quantities. It was found that G. thurberi and G. gossypioides of Section Thurberana, G. klotzschianum of Section Klotzschiana, G. anomalum of Section Anomala, G. aridum and G. harkensii of Section Erioxyla, and G. longicalyx of Section Stocksiana show the same qualitative chromatographic patterns. Their chromatograms possess the peaks of myrsitic acid, palmatic acid, stearic acid, oleic acid, and linoleic acid. These species show similar qualatative fatty acid contents. However, since morphologically and cytologically they are not closely related, the results indicate that fatty acid content is not a reliable character for classification.

G. davidsonii and G. raimondii of Section Klotzschiana, G. lobatum and G. armorianum of Section Erioxyla, and G. somalense of Section Stocksiana are chemically similar in that they all contain the palmatic acid, stearic acid, oleic acid, and linoleic acid, and lack myrsitic acid. On the basis of the chemical analyses of the fatty acids, these species

appear to be closely related. Again since G. somalense is known from cytological evidence not to be closely related to the other 4 species, the conclusion is that the fatty acid content of the oils are not reliable taxonomic characters. On the basis of the fatty acid contents of the seeds, the 12 species appear to fall into two groups. One group includes the species G. thurberi, G. gossypioides, G. aridum, G. harkensii, G. klotzschianum, G. anomalum and G. longicalyx, which contain myrsitic acid. The other group includes the species G. lobatum, G. armorianum, G. davidsonii, G. raimondii and G. somalense, which lack myrsitic acid. Since these chromatographic patterns do not follow known taxonomic relationships, the fatty acid contents of the seeds cannot be considered reliable or useful criteria of classification.

Additional samples need to be studied with each species in order to determine whether these patterns are general for each species.

Summary and Conclusion

A chromatographic analysis of the fatty acid content of the oil of 12 species of the wild lintless species of Gossypium was carried out. The species belonged to the following sections: Erioxyla, Klotzschiana, Thurberana, Anomala, and Stocksiana.

The experiment had the following objectives:

1. To determine whether there is a distinctive chromatographic pattern for each of the 12 species of the genus Gossypium.

2. To determine whether species closely related have chromatographic patterns more similar than do species which are known to be more removed.

It was not possible to find any distinct chromatographic pattern of any one of the 12 species which were under the investigation. However, if this investigation was limited to each section one can say that the

species within a section have different patterns. For instance, the species G. klotzschianum differs from the other two species of the section, G. davidsonii and G. rainmondii of Klotzschiana. The same thing was true for Stocksiana, since G. longicalyx and G. somalense show distinctive patterns. More information is needed for the other wild species which fall under Section Stocksiana which were not included in this study.

According to the chromatographic patterns of these 12 species, it was found that there are two distinctive patterns for the species of the genus Gossypium. The two groups differ by the presence or the absence of myrsitic acid C_{14}. So one can conclude that the 12 species of the genus Gossypium showed no more than two general qualitative patterns. The chemical differences between the species within the well established sections were as great as those between species in different sections. Consequently the data presented here does not lend itself to taxonomic conclusions based on biochemical differences.

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A P P E N D I X

TABLE

PERCENTAGES OF THE OIL CONTENTS OF THE TWELVE SPECIES OF THE WILD COTTONS

Species	% of the First Run	% of the Second Run	The Total %
G. thruberi	15.63	2.13	17.76
G. gossypioides	15.18	0.79	15.97
G. klotzschianum	22.83	1.96	24.79
G. davidsonii	22.42	1.14	23.56
G. raimondii	17.10	0.44	17.54
G. aridum	19.71	1.51	21.22
G. harkensii	23.90	0.56	24.46
G. lobatum	25.74	0.62	26.36
G. armorianum	19.96	0.34	20.30
G. somalense	13.02	1.20	14.22
G. longicalyx	17.70	0.71	18.41
G. anomalum	9.45	0.47	9.92

VITA

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